and dopaminergic neurons in SN and VTA. Choline acetyltransferase primers were used to identify cholinergic neurons in NB, and tryptophan hydroxylase primers were used to identify serotonergic neurons in DRN. TRPC1 mRNA was most frequently detected in all neuron types. TRPC2, involved in pheromone sensing, was not present in any neuron. TRPC3, 4 and 5 existed most frequently in cholinergic neurons in NB (80-90%). TRPC6 was relatively more frequent in dopaminergic neurons in VTA (58%) and SN (46%). TRPC7 was most frequently found in noradrenergic neurons (80%) in LC and cholinergic neurons (80%) in the NB. Interestingly, cholinergic neurons in the NB show the highest frequency of TRPC mRNA expression. Present results demonstrate that each type of neuron expresses specific combination of TRPCs, suggesting that the specific TRPCs are responsible for excitation of each type of neurons.

1368-Pos Board B212

Capsaicin Protects Mouse Neuromuscular Junctions From The Paralytic Effects Of Botulinum Neurotoxin A

Baskaran Thyagarajan, Natalia Krivitskaya, Kormakur Hognason, Joseph G. Potian, Joseph J. McArdle.
UMDNJ - NJMS, Newark, NJ, USA.

Botulinum neurotoxins (BoNTs) are the most toxic naturally occurring proteins. Botulinum serotype A (BoNT/A) selectively cleaves SNAP-25 and inhibits acetylcholine release from motor nerve endings resulting in life threatening paralysis of skeletal muscles. Here we report that capsaicin (8-methyl-N-vanillyl-6-nonenamide), an irritant active principle of chili peppers, protects against the paralytic effects of BoNT/A in mouse. In *Triangularis sterini* nerve muscle preparations, capsaicin pretreatment *in vitro*, significantly reduced fluorescent labeled BoNT/A uptake mediated by depolarization with 40 mM KCl or neural stimulations. *In vivo* injection of capsaicin (3 µl of a 1 mM stock solution) either coinjected or injected 4 or 8 hours before BoNT/A injection protected the mice from the inhibitory effects of BoNT/A measured by toe spread reflex. In controls the toe spreading reflex was inhibited within 24 hrs of poisoning with BoNT/A. Also wortmannin, a PIP5K inhibitor, injected prior to BoNT/A exposure, protected the mice from the paralytic effect of BoNT/A. *In vitro* muscle tension measurements demonstrate that capsaicin pretreatment partially protected the functions of the mouse *Extensor digitorum longus* nerve muscle preparations. Also pretreatment of cultured cholinergic neuroblastoma (N2a) cells with 10 µM capsaicin for 10 minutes prior to exposure of 10 pM BoNT/A significantly preserved labeling of synaptic vesicle pools by FM1-43. Our data collectively demonstrate that capsaicin offers protection from the paralyzing effect of BoNT/A by significantly reducing the uptake of the toxin in mouse neuromuscular junction probably by interfering with endocytosis of the toxin.

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Stretch-induced Up-regulation of Caveolae Formation and SOC Activities in HUVEC

Yuki Katanosaka, Keiji Naruse.
Okayama Univ, Okayama, Japan.

We have studied about molecular identities of stretch-activated (SA) channels and the mechanism of Ca^{2+} influx evoked by mechanical stretch in human umbilical vein endothelial cells (HUVECs). Previously, we showed that a targeting suppression of transient receptor potential 2 (TRPV2) protein expression in HUVEC using a TRPV2-specific morphorino-oligo completely blocked a transient increase of intracellular Ca^{2+} in response to stretch through the activation of SA channels. Furthermore, after these morphant HUVECs were subjected to 20% uni-axial cyclic stretch at 1 Hz for 1 h, neither a stretch-enhanced stress fiber formation nor a shift in the cell orientation transverse to the strain direction could not be observed. From these results, we concluded that TRPV2 would be a key component of SA channel complex and stretch-induced reorganization of cytoskeletons in HUVEC. Here, we examined the remodeling of Ca^{2+} responses evoked by uni-axial cyclic stretch in HUVEC. Before and after the cyclic stretch, a magnitude of single stretch-evoked Ca^{2+} transient did not change. However, the Ca^{2+} influx through the store-operated Ca^{2+} channels (SOCs) was significantly increased after stretch stimulation. Recent studies have demonstrated that caveolae are microdomains in the plasma membrane and contain functionally organized signaling molecules, including Ca^{2+} signaling. Immunohistochemistry revealed accumulation of caveolin-1 and TRPCs, some of which serve as SOCs, in caveolae after the cyclic stretch to HUVEC. Electron microscopy confirmed that the incidence of caveolae in HUVEC was increased after the stretch. On the other hands, TRPV2-knocked down HUVECs suppressed the increased SOC activities and caveolae formation after cyclic stretch. Such the up-regulation of SOC activities through stretch-dependent TRPV2 activation might contribute to sustained intracellular Ca^{2+} increase, which is thought to be a primary etiology of the vascular remodeling, and a potent risk factor of pressure-dependent hypertrophic diseases.

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Regulatory Elements of TRPA1 Function

Barbara Lackner[1], Reinhard Fritsch[1], Irene Frischauf[1], Rainer Schindl[1], Klaus Groschner[2], Christoph Romanin[1].
[1]University of Linz, Linz, Austria, [2]University of Graz, Graz, Austria.

Transient Receptor Potential Ankyrin 1 channel, TRPA1, is a member of the TRP family and has been shown to be expressed in dorsal root ganglions, trigeminal ganglion neurons and hair cells. TRPA1 is proposed to play an important role in pain perception and temperature sensing, and might be involved as transduction channel in mechanosensation essential for the auditory response in mammals. Pungent chemicals and environmental irritants (e.g. mustard oil) are substances activating this ion channel. Specifically, activation of TRPA1 by allyl isothiocyanates occurs via covalent modification of cysteine residues within the cytoplasmic N terminus of the channel. Here we have focused on the role of TRPA1 C-terminus employing Ca^{2+} imaging, patch-clamp and confocal fluorescence resonance energy transfer (FRET) microscopy. A TRPA1 C-terminal deletion mutant failed to activate upon allyl isothiocyanate application despite a similar homomerization potential as the wild-type form evident from both functional assays and FRET measurements, respectively. The TRPA1 C-terminus alone exhibited multimerization potential in FRET microscopy, yet failed to function as a dominant negative species when co-expressed with wild-type TRPA1 suggesting a minor role for channel assembly. Further, a single amino acid mutation in TRPA1 C-terminus led to a substantial reduction in TRPA1 currents. With regard to a potential regulatory role of calmodulin (CaM), FRET experiments suggested its association with TRPA1 upon elevation of intracellular calcium concentrations either by allyl isothiocyanate induced stimulation of TRPA1 or ionomycin. Consistently, electrophysiological experiments revealed faster inactivation of TRPA1 in presence of over-expressed CaM. In summary our experiments point to TRPA1 C-terminus a crucial element for channel function that is additionally regulated by CaM. (supported by FWF P18169)